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TOXICITY OF NICKEL TO MICROORGANISMS IN SOIL: INFLUENCE OF SOME PHYSICOCHEMICAL CHARACTERISTICS

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ABSTRACT

The tolerance to nickel (Ni) in soil varied among a variety of eubacteria, actinomycetes, yeasts, and filamentous fungi. The incorporation of 12% montmorillonite and, to a lesser extent, of 12% kaolinite reduced the toxicity of Ni to mycelial extension of filamentous fungi. The ability of the clays to protect against Ni toxicity was probably related to their cation exchange capacity (CEC). The toxicity of Ni to mycelial extension of filamentous fungi was also reduced when the pH of a naturally acidic (pH 4·9) soil was raised to a neutral pH. The toxicity of 0 to 1000 ppm Ni to eubacteria, actinomycetes, and yeasts was completely eliminated in a naturally alkaline soil (pH 7·7) containing a montmorillonite-type clay and a CEC of 16 meq 100 g^{-1}, whereas the same concentrations of Ni were toxic in a naturally acidic soil that lacked montmorillonite-type clays and had a CEC of only 8 meq 100 g^{-1}.

INTRODUCTION

Increased industrialization has resulted in the contamination of many aquatic and terrestrial ecosystems by nickel (Ni). Common sources of environmental contamination by Ni include smelting and mining operations, the manufacture of alloys, alkaline storage batteries, and electronic components, the application of commercial fertilizers, the use of sewage sludge as landfill material, and the combustion of fossil fuels (Nriagu, 1980). Although there has been some research on the toxicity of Ni to microbes exposed in synthetic media (Kaltwasser & Friggs, 1980; Spencer, 1980), few studies have determined the toxicity of Ni to microbes in natural ecosystems. Nickel has been shown to inhibit nitrification (Liang & Tabatabai, 1978), carbon mineralization (Bhuiya & Cornfield, 1972; Cornfield, 1977), and the activities of acid and alkaline phosphatases (Juma & Tabatabai,

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1977) and arylsulphatase (Al-Khafaji & Tabatabai, 1979) in soil and to inhibit decomposition of forest lead litter (Spalding, 1979; Freedman & Hutchinson, 1980).

The toxicity of heavy metals (Babich & Stotzky, 1980), including that of Ni, to microorganisms is influenced by the physicochemical properties of the synthetic laboratory medium or of the recipient natural ecosystem. In synthetic media, the toxicity of Ni to microbes was decreased by increasing the hardness (as $CaCO_3$) component (Babich & Stotzky, 1981), the concentration of chelating agents (Ainsworth *et al.*, 1980), the pH from acidic to alkaline levels (Babich & Stotzky, 1982*a*), or the magnesium content (Abelson & Aldous, 1950). However, limited research has evaluated this modifying influence of abiotic factors on Ni toxicity to microbes in natural ecosystems. Increasing the hydrostatic pressure potentiated the toxicity of Ni to marine bacteria characterized as reducers or oxidizers of manganese (Mn) (Arcuri & Ehrlich, 1977), and the survival of *Rhodotorula rubra* after 35 days of exposure to 10 ppm Ni was greater in lake water (pH 6·8) amended with 200 or 400 mg litre^{-1} $CaCO_3$ than in the same lake water also amended with 10 ppm Ni and with a natural background level of 34 mg litre^{-1} $CaCO_3$ (Babich & Stotzky, details to be published later). The pH affected the toxicity of Ni towards carbon and nitrogen mineralization in soils. Nitrification was decreased to a greater extent with increasing levels of Ni (from 50 to 5000 ppm) at pH 5·8 than at pH 6·9 and 7·6, whereas nitrogen mineralization was decreased to about the same extent with increasing levels of Ni at all pH levels. Carbon mineralization was decreased with increasing levels of Ni to about the same extent at pH 5·8 and 6·9 but to a lesser extent at pH 7·6 (Giashuddin & Cornfield, 1979).

As there is comparatively little information on the toxicity of Ni to microbes in soil and on the influence of abiotic factors on Ni toxicity in soil, studies were conducted to determine (1) the levels of Ni that are inhibitory to representative microbes in soil; (2) the influence of clay minerals and pH on Ni toxicity in soil; and (3) the differential toxicity of Ni in natural soils that differ in their physicochemical characteristics (i.e., an acidic soil with a low cation exchange capacity (CEC) as compared with an alkaline soil with a higher CEC).

MATERIALS AND METHODS

Description of soils

Soil, obtained from the Kitchawan Research Laboratory of the Brooklyn Botanic Garden at Ossining, NY, was amended with either montmorillonite (mont.) (Volclay, Panther Creek, American Colloid Co., with a CEC of approximately 60 meq 100 g^{-1}) or kaolinite (kaol.) (Continental, R.T. Vanderbilt Co., with a CEC of approximately 6·5 meq 100 g^{-1}) to a concentration of 12% (v/v). X-ray diffraction analysis of the unamended soil showed that it does not contain montmorillonite-type clay minerals but does contain mica-illite, kaolinite, and

TABLE 1

SOME CHEMICAL AND PHYSICAL CHARACTERISTICS OF KITCHAWAN SOIL, KITCHAWAN SOIL AMENDED WITH EITHER 12% KAOLINITE (kaol.) OR 12% MONTMORILLONITE (mont.), AND MOPALA SOIL

Characteristic	*Soil*			
	Kitchawan	*Kitchawan + 12% kaol.*	*Kitchawan + 12% mont.*	*Mopala*
pH	4·9	4·8	5·6	7·7
Organic matter (%)	5·75	4·71	5·13	1·8
Cation exchange capacity (meq 100 g^{-1})	8·15	9·61	14·67	16·0
1/3-Bar tension water content (%)	20·7	22·0	24·4	20·0
Sand (%)	56·8	52·8	55·8	53·0
Silt (%)	33·8	27·6	26·6	29·0
Clay (%)	9·4	19·6	17·6	18·0
P (ppm)	27·6	32·4	43·4	20·0
K (ppm)	61·9	57·2	107·6	825·0
Ca (ppm)	360·0	409·0	2028·0	7225·0
Mg (ppm)	63·1	62·4	140·4	225·0
Na (ppm)	3·9	8·2	14·1	ND
Mn (ppm)	5·8	7·7	5·4	ND
Fe (ppm)	1·5	1·0	0·9	ND
Cu (ppm)	13·24	ND	ND	262·33
Pb (ppm)	24·57	ND	ND	13·94
Zn (ppm)	78·84	ND	ND	87·03
Cd (ppm)	0·01	ND	ND	0·19
Cr (ppm)	12·66	ND	ND	11·90
Ni (ppm)	19·73	ND	ND	16·73

ND, not determined (*see* Babich & Stotzky, 1977, 1978).

vermiculite (Babich & Stotzky, 1977). In some experiments, the pH of this soil was raised from 4·9 to 7·1 by the addition of 1·3% $CaCO_3$. Soil from Mopala farm, a banana plantation in La Lima, Honduras, was also used. The Mopala soil is an alkaline soil (pH 7·7) that naturally contains illite, kaolonite, and montmorillonite-type clays (Stotzky & Martin, 1963).

Some of the physical and chemical characteristics of the Kitchawan soil, unamended and amended with clays, and of the Mopala soil are presented in Table 1. The methodologies for these analyses (most of which were performed through the courtesy of the late Dr A. L. Leaf) were as follows: soil texture was determined by the hydrometer method, organic matter by loss on ignition at approximately 480 °C for 16 h, and CEC by saturating the exchange capacity with 1 N ammonium acetate, washing out the excess ammonium (NH_4^+) with alcohol, replacing the NH_4^+ with potassium (K^+) from 1 N acidified potassium chloride, and distilling off the NH_4^+ by the macrokjeldahl procedure. Phosphorus (P) was extracted by saturating with 0·002 N sulphuric acid followed by colorimetric determinations using ammonium molybdate–stannous chloride. Potassium, calcium (Ca), magnesium (Mg), sodium (Na), manganese (Mn), and iron (Fe) were extracted with 1 N neutral ammonium acetate and analysed by atomic absorption spectrophotometry. Determinations of

the levels of Ni, cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), and zinc (Zn) were performed (through the courtesy of Dr T. Kneip) by atomic absorption spectrophotometry following sequential extraction with water and nitric acid. All pH values are for a 1:2 soil:water suspension and the 1/3 bar tension water content was determined with a 5-bar pressure plate extractor (Soilmoisture Equipment Corp., Santa Barbara, Calif.).

Experimental design

Filamentous fungi. The soil-replica plating technique (Stotzky, 1965, 1973) was used to study the growth rates (i.e., mycelial extension per day) of fungi in the soils unamended or amended with clays, $CaCO_3$, and Ni, as $NiCl_2.6H_2O$. Soil was placed in polyethylene bags, and water was added to bring the soil to 2 % above the 1/3-bar tension water content, where respiration and microbial activity of soil are at an optimum (Stotzky, 1972). In soils amended with $CaCO_3$ or Ni, the salts were incorporated into the water. The soil and water were mixed thoroughly, stored overnight at 4 °C, passed through a 2-mm sieve, and 35 to 40 g of soil, retained by a 0·5 mm sieve, was dispensed into heavy-walled petri dishes and levelled. The soil plates were autoclaved for 15 min (121 °C at 15 psi), during which time the extra 2 % water was lost and the sterilised soils were at their 1/3-bar tension water content.

Slants containing fungi grown for 1 week at 25 °C on Sabouraud dextrose agar (SDA) were flooded with 0·85 % saline and agitated on a Vortex Genie. An 0·1 ml inoculum of the suspension, which consisted primarily of spores but also contained some hyphae, was placed in a central depression, marked before autoclaving with the aid of the template, in the sterilized soil plates. After inoculation, the soil plates were placed in a humidifier-incubator maintained at 25 °C. Three replicate soil plates were used for each concentration of Ni and each environmental variable, and experiments were performed at least twice.

Soil plates of *Rhizopus stolonifer* and *Trichoderma viride* were replicated on days 1 and 5 after inoculation, and soil plates of *Aspergillus flavus*, *Aspergillus flavipes*, *Aspergillus clavatus*, *Aspergillus niger*, *Penicillium vermiculatum*, and *Gliocladium* sp. were replicated on days 1 and 8 after inoculation. Replications were made to SDA amended with 33·3 mg litre^{-1} Rose Bengal. Replicated agar plates were incubated at 25 °C for several days, and measurements of the diameter of spread (i.e., mycelial extension) in soil were made in four directions. Maps were constructed showing the spread of the fungi in different soils, and based on these maps, growth rates were calculated. Replications on day 1 were used to determine the initial diameter of the inoculum in the soil. Growth rates, in mm day^{-1}, were calculated by dividing the diameter of fungal spread at the termination of the experiment, minus the initial diameter of the inoculum, by the number of days of growth.

Unicellular microbes. Eubacteria (*Agrobacterium radiobacter*, *Serratia marcescens*, *Proteus vulgaris*, *Bacillus cereus*, *Bacillus megaterium*), an actinomycete (*Nocardia rhodochrous*), and yeasts (*Cryptococcus terreus*, *Rhodotorula rubra*, *Torulopsis glabrata*) were grown in tubes containing nutrient broth (Difco) amended with 1 % glucose and 0·1 % yeast extract and placed for 18 h in a rotating drum (36 rpm) housed in a 25 °C incubator. The cells were washed twice with 0·85 % saline, resuspended in saline to yield a percent transmittance of 80 to 85 (B & L Spectronic 20), and 0·5 ml of the microbial suspension was inoculated into tubes containing 1 g autoclaved dry soil unamended or amended with Ni (this brought the soils to approximately twice their 1/3-bar tension water content). The tubes were incubated for 1 week at 25 °C, 2 ml saline was added, the tubes were agitated on a Vortex Genie, and the soils were serially diluted in saline and pour plates were made with nutrient agar (Difco) amended with 1 % glucose and 0·1 % yeast extract. The plates were inverted, incubated at 25 °C for 1 to 3 days, and colonies were counted. Three replicate soil tubes were used for each concentration of Ni, and experiments were performed twice.

All organisms were obtained from the culture collection of the Laboratory of Microbial Ecology, Biology Department, New York University.

Statistics. The data are expressed as the arithmetic mean ± the standard error of the mean, and the Student's *t* test was performed, with $P < 0{\cdot}05$ being considered significant.

RESULTS

Incipient inhibition of growth in Kitchawan soil by Ni (i.e., the level of added Ni at which statistically significant inhibition of growth was noted initially) occurred at 50 ppm for *A. clavatus*, at 100 ppm for *P. vermiculatum*, at 250 ppm for *A. flavus* and *Gliocladium* sp., at 500 ppm for *R. stolonifer*, *A. flavipes*, and *A. niger*, and at 750 ppm for *T. viride*. In soil amended with 1000 ppm Ni, there was no growth of *A. flavipes*, *A. flavus*, *A. clavatus*, *A. niger*, and *P. vermiculatum*, limited growth of *R. stolonifer* and *T. viride*, and moderate growth of *Gliocladium* sp. (Table 2).

The toxicity of 750 ppm Ni to *A. flavipes*, *A. clavatus*, and *Gliocladium* sp. was reduced in Kitchawan soil amended with 12 % kaol. when compared with the extent of growth reduction in Kitchawan soil not amended with clay but supplemented with 750 ppm Ni. There were no statistically significant differences between unamended and kaol.-amended soils in the extent of Ni toxicity to *R. stolonifer*, *P. vermiculatum*, and *T. viride*. An equivalent amount of mont. provided greater protection than did kaol. against the toxicity of Ni: the toxicity of Ni towards *Gliocladium* sp. and *R. stolonifer* was completely eliminated and greatly reduced towards *A. flavipes*, *A. clavatus*, *P. vermiculatum*, and *T. viride* (Table 3).

The toxicity of 1000 ppm Ni to growth of the fungi was reduced in Kitchawan soil adjusted to approximately pH 7·0. *P. vermiculatum* exhibited no growth, and *R. stolonifer*, *T. viride*, and *Gliocladium* sp. exhibited only limited to moderate growth when the naturally acidic soil (pH < 5) was amended with 1000 ppm Ni. However, in

TABLE 2

GROWTH OF FILAMENTOUS FUNGI IN KITCHAWAN SOIL AMENDED WITH PROGRESSIVELY INCREASING CONCENTRATIONS OF Ni[a]

Treatment (Ni, in ppm)	*Mycelial growth rates, in mm day^{-1}[b] (% of control)[c]*							
	Aspergillus flavipes	Aspergillus flavus	Aspergillus clavatus	Aspergillus niger	Penicillium vermiculatum	Rhizopus stolonifer	Trichoderma viride	Gliocladium *sp.*
No Ni added	9·5 ± 0·56	6·0 ± 0·26	7·4 ± 0·34	4·0 ± 0·16	4·9 ± 0·08	13·1 ± 0·20	11·0 ± 0·16	4·4 ± 0·16
	(100 ± 5·9)	(100 ± 4·3)	(100 ± 4·5)	(100 ± 3·9)	(100 ± 1·6)	(100 ± 1·6)	(100 ± 0·3)	(100 ± 3·7)
10	8·7 ± 0·28	5·5 ± 0·31	7·6 ± 0·48	4·0 ± 0·34	4·7 ± 0·12	12·7 ± 0·29	11·1 ± 0·00	4·6 ± 0·13
	(92 ± 2·9)	(92 ± 5·2)	(103 ± 6·5)	(100 ± 8·5)	(96 ± 2·5)	(97 ± 2·2)	(101 ± 0·0)	(105 ± 3·0)
50	9·2 ± 0·23	5·9 ± 0·11	4·7 ± 0·44[d]	4·0 ± 0·45	4·6 ± 0·12	12·3 ± 0·27	11·1 ± 0·00	4·4 ± 0·13
	(97 ± 2.4)	(98 ± 1·8)	(64 ± 5·9)	(100 ± 11.3)	(96 ± 2·5)	(94 ± 2·1)	(101 ± 0·0)	(100 ± 3·2)
100	9·3 ± 0·13	5·4 ± 0·13	3·1 ± 0·31[d]	3·4 ± 0·32	4·5 ± 0·08	12·4 ± 0·22	11·1 ± 0·00	4·4 ± 0·13
	(98 ± 1·4)	(90 ± 2·2)	(41 ± 4·2)	(85 ± 8·0)	(92 ± 1·6)	(95 ± 1·7)	(101 ± 0·0)	(100 ± 3·2)
250	9·4 ± 0·11	4·2 ± 0·08[d]	1·5 ± 0·21[d]	3·5 ± 0·53	2·9 ± 0·47[d]	12·5 ± 0·29	11·0 ± 0·04	3·7 ± 0·07[d]
	(99 ± 1·2)	(70 ± 1·3)	(20 ± 2·8)	(88 ± 13·3)	(59 ± 9·6)	(96 ± 2·2)	(100 ± 0·4)	(84 ± 1·6)
500	0·8 ± 0·06[d]	4·0 ± 0·36[d]	1·1 ± 0·17[d]	2·3 ± 0·43[d]	2·4 ± 0·30[d]	2·1 ± 0·07[d]	10·6 ± 0·33	3·4 ± 0·13[d]
	(8 ± 0·6)	(67 ± 6·0)	(15 ± 2·3)	(58 ± 10·1)	(48 ± 6·2)	(16 ± 5·1)	(96 ± 0·3)	(77 ± 3·0)
750	1·0 ± 0·22[d]	1·4 ± 0·18[d]	0·7 ± 0·14[d]	0·2 ± 0·06[d]	1·5 ± 0·26[d]	1·5 ± 0·47[d]	1·6 ± 0·37[d]	2·4 ± 0·06[d]
	(11 ± 2·3)	(24 ± 3·0)	(9 ± 1·9)	(5 ± 1·5)	(30 ± 5·4)	(11 ± 3·6)	(15 ± 3·4)	(55 ± 1·4)
1000	0[d]	0[d]	0[d]	0[d]	0·4 ± 0·12[d]	0·9 ± 0·25[d]	1·3 ± 0·36[d]	2·1 ± 0·24[d]
	(0)	(0)	(0)	(0)	(1 ± 0·3)	(7 ± 1·9)	(12 ± 3·3)	(48 ± 5·5)

[a] Kitchawan soil is acidic (pH 4·9) with a CEC of 8·15 meq 100 g^{-1}. The pH values of the soils amended with Ni were: pH 4·9—10 ppm Ni; pH 4·8—50 & 100 ppm Ni; pH 4·7—250 ppm Ni; pH 4·6—500 ppm Ni; pH 4·5—750 & 1000 ppm Ni.
[b] Mean ± standard error of the mean.
[c] Control soil contained no Ni.
[d] Statistically significant at $P < 0·05$ when compared with control soil.

TABLE 3

INFLUENCE OF KAOLINITE (kaol.) AND MONTMORILLONITE (mont.) ON THE TOXICITY OF NICKEL TO THE GROWTH OF FUNGI IN KITCHAWAN SOIL

Treatment	*Soil pH*	*C.E.C. (meq 100 g)*	*Mycelial growth rates, in mm/day[a] (% of control)[b]*					
			Aspergillus flavipes	Aspergillus clavatus	Rhizopus stolonifer	Penicillium vermiculatum	Trichoderma viride	Gliocladium *sp.*
No. Ni added	4·8	8·15	6·7 ± 0·42	5·7 ± 0·43	5·0 ± 0·45	6·2 ± 0·76	7·4 ± 0·76	6·6 ± 0·29
			(100 ± 6·2)	(100 ± 7·6)	(100 ± 9·0)	(100 ± 8·2)	(100 ± 10·2)	(100 ± 4·4)
750 ppm Ni	4·7		0·5 ± 0·11[c]	0·9 ± 0·20[c]	1·1 ± 0·27[c]	1·1 ± 0·27[c]	1·1 ± 0·27[c]	3·1 ± 0·35[c]
			(7 ± 1·6)	(16 ± 3·6)	(22 ± 12·6)	(18 ± 3·6)	(15 ± 3·6)	(48 ± 5·3)
12% kaol.	4·8	9·61	5·5 ± 0·75	5·1 ± 0·25	4·7 ± 0·61	7·4 ± 0·40	7·4 ± 0·40	4·5 ± 0·13
			(100 ± 13·8)	(100 ± 4·8)	(100 ± 12·9)	(100 ± 7·2)	(100 ± 6·4)	(100 ± 3·0)
12% kaol. + Ni	4·7		2·0 ± 0·56[c]	1·8 ± 0·19[c]	2·3 ± 0·60[c]	0·8 ± 0·20[c]	1·9 ± 0·36[c]	4·8 ± 0·24
			(36 ± 0·2)[d]	(36 ± 3·8)[d]	(46 ± 12·7)	(11 ± 3·6)	(27 ± 4·9)	(107 ± 5·0)[d]
12% mont.	5·6	14·67	5·0 ± 0·36	6·1 ± 0·50	4·4 ± 0·61	5·6 ± 0·56	8·6 ± 0·21	6·3 ± 0·42
			(100 ± 7·2)	(100 ± 8·3)	(100 ± 13·9)	(100 ± 9·9)	(100 ± 2·4)	(100 ± 6·7)
12% mont. + Ni	5·6		2·8 ± 0·22[c]	4·2 ± 0·25[c]	3·7 ± 0·47	4·1 ± 0·30[c]	5·2 ± 0·75[c]	6·1 ± 0·25
			(56 ± 4·4)[d]	(69 ± 4·1)[d]	(84 ± 10·7)[d]	(73 ± 5·4)[d]	(60 ± 8·7)[d]	(97 ± 4·0)[d]

[a] Mean ± standard error of the mean.
[b] Control soil did not contain Ni.
[c] Statistically significant at $P < 0·05$ when compared with respective soil mixtures without Ni.
[d] Statistically significant at $P < 0.05$ when compared with soil mixtures amended with Ni but not clay.

TABLE 4

GROWTH OF FILAMENTOUS FUNGI IN KITCHAWAN SOIL, UNADJUSTED AND ADJUSTED TO NEUTRAL pH, IN THE ABSENCE AND PRESENCE OF 1000 ppm Ni

Treatment	*pH*	*Mycelial growth rates, in mm day*$^{-1}$ [a] (*% of control*)[b]			
		Penicillium vermiculatum	Rhizopus stolonifer	Trichoderma viride	Gliocladium *sp.*
No Ni added; no $CaCO_3$ added	4·9	4·6 ± 0·13 (100 ± 2·9)	9·1 ± 0·48 (100 ± 5·2)	8·9 ± 0·37 (100 ± 4·1)	5·2 ± 0·12 (100 ± 2·4)
1000 ppm Ni; no $CaCO_3$ added	4·7	0[c] (0)	1·3 ± 0·37[c] (14 ± 4·1)	0·8 ± 0·25[c] (9 ± 2·8)	0·9 ± 0·09[c] (17 ± 1·7)
No Ni added; 1·3% $CaCO_3$ added	7·1	3·5 ± 0·16 (100 ± 4·5)	8·7 ± 0·38 (100 ± 4·4)	9·0 ± 0·29 (100 ± 3·2)	5·0 ± 0·14 (100 ± 2·7)
1000 ppm Ni; 1·3% $CaCO_3$ added	6·9	2·4 ± 0·15[c] (69 ± 4·3)[d]	5·1 ± 0·48[c] (59 ± 5·5)[d]	3·9 ± 0·55[c] (43 ± 6·1)[d]	2·3 ± 0·21[c] (45 ± 4·2)[d]

[a] Mean ± standard error of the mean.
[b] Control soil contained no added Ni and an equivalent concentration of $CaCO_3$.
[c] Statistically significant at $P < 0·05$ when compared with respective soil mixtures without Ni.
[d] Statistically significant at $P < 0·05$ when compared with soil mixtures amended with Ni but not with $CaCO_3$

soil adjusted to approximately a neutral pH, the same level of Ni was only about 50% inhibitory (Table 4).

Incipient decrease in survival (i.e., the level of added Ni at which a statistically significant decrease in viability was noted initially) of unicellular microbes in Ni-amended Kitchawan soil occurred at 250 ppm Ni for *A. radiobacter*, *B. megaterium*, *C. terreus*, and *T. glabrata*, at 500 ppm Ni for *S. marcescens*, and at 750 ppm Ni for *P. vulgaris*, *B. cereus*, *N. rhodochrous*, and *R. rubra* after 1 week of exposure. The viability of *A. radiobacter* and *B. megaterium* was completely reduced at 500 ppm Ni and that of *S. marcescens* at 1000 ppm Ni. *P. vulgaris*, *B. cereus*, *N. rhodochrous*, *C. terreus*, *R. rubra*, and *T. glabrata* exhibited some viability even at 1000 ppm Ni (Table 5). In Mopala soil amended with equivalent concentrations of Ni, levels of up to 1000 ppm Ni did not adversely affect the viability of *A. radiobacter*, *S. marcescens*, *B. megaterium*, *N. rhodochrous*, *C. terreus*, and *R. rubra* (Table 6).

DISCUSSION

Concentrations of Ni that were inhibitory to growth (i.e., colony expansion) of filamentous fungi and to the growth and survival of yeasts, eubacteria, and an actinomycete in the Kitchawan soil were similar to those reported by others who also studied the toxicity of Ni in acidic soils. For example, 25 to 100 ppm Ni decreased the number of fungi isolated (Freedman & Hutchinson, 1980), 100 (Cornfield, 1977) to 1000 ppm (Bhuiya & Cornfield, 1972) Ni decreased carbon mineralization, and 50 to 5000 ppm Ni inhibited ammonification and nitrification (Giashuddin & Cornfield, 1979) in soil.

The decreases in the toxicity of Ni to filamentous fungi by the addition of mont. and, to a lesser extent, of kaol. to the Kitchawan soil were similar to the decreases

TABLE 5

GROWTH AND SURVIVAL OF MICROORGANISMS IN KITCHAWAN SOIL UNAMENDED AND AMENDED WITH PROGRESSIVELY INCREASING CONCENTRATIONS OF Ni[a]

Organism	*Colony-forming units per g of soil after 7 days of exposure*[b]						
	Initial	*Nickel (ppm)*					
		0	*250*	*500*	*750*	*1000*
Agrobacterium radiobacter	$2·8 \pm 1·11 \times 10^7$	$1·3 \pm 0·34 \times 10^7$	$2·0 \pm 0·92 \times 10^{5c}$	0[c]	0[c]	0[c]
Serratia marcescens	$1·6 \pm 0·14 \times 10^6$	$3·2 \pm 0·17 \times 10^7$	$2·7 \pm 0·17 \times 10^7$	$4·0 \pm 1·00 \times 10^{6c}$	$1·2 \pm 0·70 \times 10^{3c}$	0[c]
Proteus vulgaris	$8·0 \pm 3·17 \times 10^4$	$5·9 \pm 1·71 \times 10^2$	$2·7 \pm 0·38 \times 10^2$	$2·2 \pm 0·24 \times 10^2$	$1·4 \pm 0·15 \times 10^{2c}$	$1·3 \pm 0·14 \times 10^{2c}$
Bacillus megaterium	$1·0 \pm 0·15 \times 10^5$	$3·1 \pm 1·35 \times 10^4$	$6·0 \pm 0·58 \times 10^{1c}$	0[c]	0[c]	0[c]
Bacillus cereus	$1·0 \pm 0·34 \times 10^4$	$6·5 \pm 0·39 \times 10^3$	$6·2 \pm 0·45 \times 10^3$	$5·4 \pm 0·54 \times 10^3$	$4·4 \pm 0·33 \times 10^3$	$4·6 \pm 0·27 \times 10^{3c}$
Nocardia rhodochrous	$4·6 \pm 0·66 \times 10^6$	$6·8 \pm 1·09 \times 10^6$	$6·8 \pm 1·14 \times 10^6$	$5·1 \pm 0·37 \times 10^6$	$3·6 \pm 0·38 \times 10^{6c}$	$2·8 \pm 0·71 \times 10^{6c}$
Cryptococcus terreus	$4·4 \pm 0·49 \times 10^4$	$1·1 \pm 0·15 \times 10^5$	$3·1 \pm 0·53 \times 10^{4c}$	$2·8 \pm 0·59 \times 10^{3c}$	$2·0 \pm 0·37 \times 10^{3c}$	$1·7 \pm 0·30 \times 10^{3c}$
Rhodotorula rubra	$5·7 \pm 0·61 \times 10^5$	$1·2 \pm 0·06 \times 10^6$	$1·0 \pm 0·08 \times 10^6$	$1·0 \pm 0·09 \times 10^6$	$8·5 \pm 1·00 \times 10^{5c}$	$7·1 \pm 0·88 \times 10^{5c}$
Torulopsis glabrata	$1·1 \pm 0·17 \times 10^5$	$1·0 \pm 0·15 \times 10^6$	$1·6 \pm 0·11 \times 10^{5c}$	$4·0 \pm 0·40 \times 10^{4c}$	$1·1 \pm 0·26 \times 10^{4c}$	$6·4 \pm 2·38 \times 10^{3c}$

[a] Kitchawan soil is an acidic soil (pH 4·9) with a CEC of 8·15 meq 100 g. The pH values of the soils amended with Ni are pH 4·7—250 ppm Ni; pH 4·6—500 ppm Ni; and pH 4·5—750 and 1000 ppm Ni.
[b] Mean ± standard error of the mean.
[c] Statistically significant at $P < 0·05$ when compared with control soil not amended with Ni and after 7 days of incubation.

TABLE 6

GROWTH AND SURVIVAL OF MICROORGANISMS IN MOPALA SOIL UNAMENDED AND AMENDED WITH PROGRESSIVELY INCREASING CONCENTRATIONS OF Ni[a]

Organism	Colony-forming units per g of soil[b] after 7 days of exposure						
			Nickel (ppm)				
	Initial	0	250	500	750	1000
Agrobacterium radiobacter	$4{\cdot}2 \pm 1{\cdot}14 \times 10^7$	$7{\cdot}2 \pm 0{\cdot}75 \times 10^7$	$6{\cdot}5 \pm 0{\cdot}50 \times 10^7$	$6{\cdot}1 \pm 0{\cdot}68 \times 10^7$	$6{\cdot}8 \pm 0{\cdot}91 \times 10^7$	$6{\cdot}2 \pm 0{\cdot}62 \times 10^7$
Serratia marcescens	$1{\cdot}6 \pm 0{\cdot}32 + 10^7$	$3{\cdot}2 \pm 0{\cdot}22 \times 10^7$	$3{\cdot}4 \pm 0{\cdot}31 \times 10^7$	$3{\cdot}3 \pm 0{\cdot}53 \times 10^7$	$3{\cdot}0 \pm 0{\cdot}51 \times 10^7$	$2{\cdot}7 \pm 0{\cdot}33 \times 10^7$
Bacillus megaterium	$2{\cdot}2 \pm 0{\cdot}31 \times 10^4$	$2{\cdot}3 \pm 0{\cdot}19 \times 10^4$	$2{\cdot}0 \pm 0{\cdot}16 \times 10^4$	$2{\cdot}0 \pm 0{\cdot}16 \times 10^4$	$2{\cdot}0 \pm 0{\cdot}21 \times 10^4$	$1{\cdot}8 \pm 0{\cdot}13 \times 10^4$
Nocardia rhodochrous	$7{\cdot}4 \pm 0{\cdot}42 \times 10^6$	$1{\cdot}5 \pm 0{\cdot}06 \times 10^6$	$1{\cdot}4 \pm 0{\cdot}08 \times 10^6$	$1{\cdot}3 \pm 0{\cdot}06 \times 10^6$	$1{\cdot}4 \pm 0{\cdot}04 \times 10^6$	$1{\cdot}3 \pm 0{\cdot}06 \times 10^6$
Cryptococcus terreus	$4{\cdot}5 \pm 0{\cdot}48 \times 10^6$	$5{\cdot}7 \pm 1{\cdot}36 \times 10^6$	$5{\cdot}4 \pm 1{\cdot}36 \times 10^6$	$6{\cdot}0 \pm 1{\cdot}48 \times 10^6$	$5{\cdot}3 \pm 1{\cdot}32 \times 10^6$	$4{\cdot}5 \pm 1{\cdot}20 \times 10^6$
Rhodotorula rubra	$1{\cdot}8 \pm 0{\cdot}47 \times 10^7$	$2{\cdot}4 \pm 0{\cdot}42 \times 10^7$	$2{\cdot}5 \pm 0{\cdot}34 \times 10^7$	$2{\cdot}4 \pm 0{\cdot}40 \times 10^7$	$2{\cdot}5 \pm 0{\cdot}41 \times 10^7$	$2{\cdot}5 \pm 0{\cdot}43 \times 10^7$

[a] Mopala soil is an alkaline soil (pH 7·7) with a CEC of 16 meq 100 g^{-1}. The pH values of the soils amended with Ni are: pH 7·6—250 ppm Ni; pH 7·5—500, 750, and 1000 ppm Ni.
[b] Mean ± standard error of the mean.

obtained in the toxicity of cadmium (Cd) in this clay-amended soil (Babich & Stotzky, 1977). The cationic forms of Ni (Heinrichs & Mayer, 1980) and other heavy metals (Farrah & Pickering, 1977; Babich & Stotzky, 1980) sorb to the negatively-charged exchange sites on clay minerals and other negatively-charged particulates present in terrestrial and aquatic ecosystems. The incorporation of mont. and kaol. into the Kitchawan soil increased its CEC and, therefore, the ability of this soil to sorb Ni, which, in turn, apparently reduced the availability of the Ni for uptake by the microbiota. The greater protection against the toxicity of Ni in the mont.-amended soil was probably related to the higher CEC of this soil mixture as compared with the kaol.-amended or unamended Kitchawan soil.

The decrease in the toxicity of Ni to the growth of filamentous fungi in Kitchawan soil whose pH was increased from an acidic to a neutral level was probably the result of the more efficient competition between Ni^{2+} and protons (H^+) for exchange sites on the inorganic and organic particulates in the soil: i.e., as the pH was increased, there were less H^+ to compete with Ni^{2+} for exchange sites and therefore the effective concentration of Ni^{2+} in the soil solution was decreased. The formation at the higher pH level of Ni complexes with components in the soil solution, with these complexes being less toxic than free Ni, may have also been involved in the reduction in Ni toxicity. However, the inverse relation between pH and Ni toxicity cannot be attributed to the formation of various hydroxylated species of Ni (i.e., $NiOH^+$, $Ni(OH)_2$, $Ni(OH)_3^-$, $Ni(OH)_4^{2-}$) that might have different toxicities (as has been shown for other heavy metals (Babich & Stotzky, 1980)), as these hydroxylated species only form in appreciable amounts at pH 9 and above (Richter & Theis, 1980), i.e., at pH levels higher than those employed in these studies. The inverse relation between pH and Ni toxicity in soil confirms observations in synthetic media, where increases in the pH of the media decreased the toxicity of Ni to bacteria (freshwater, marine, and terrestrial) and fungi (terrestrial) (Babich & Stotzky, 1982*a*). The toxicity of Ni to carbon mineralization and nitrification in soil was also lower in alkaline than in acidic soil (Giashuddin & Cornfield, 1979).

Although 1000 ppm Ni was either inhibitory or lethal to eubacteria, an actinomycete, and yeasts in the Kitchawan soil, a comparable level of Ni in the Mopala soil was not toxic. The higher pH and CEC of the Mopala soil were probably the primary abiotic factors responsible for reducing the toxicity of Ni. However, the higher level of Mg in the Mopala soil may have contributed to the reduction in Ni toxicity, as Mg has been shown to reduce the uptake of Ni by, and hence its toxicity to, bacteria, yeasts, and filamentous fungi (Abelson & Aldous, 1950). The reduction in the toxicity of Ni to estuarine/marine fungi that occurred with increases in the concentration of sea salts or seawater was also attributed to the higher Mg content (Babich & Stotzky, 1982*b*).

The apparent mediating influence of alkaline pH, type and amount of clay minerals and, possibly, the Mg content on the toxicity of Ni to microbes in soil demonstrates the need for regulatory agencies to consider these and other abiotic

factors when formulating standards for environmental toxicants (Babich & Stotzky, 1980, Babich *et al.*, 1981). A standard for a toxicant, such as Ni, that is uniform for all environments, irrespective of their differing physicochemical characteristics and the subsequent effects of these differences on pollutant toxicity, is not adequate, as such a standard would be over-protective for some ecosystems and under-protective for others.

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